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Tibial subchondral bone mineral density: sources of variability and reproducibility

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SUMMARY

Objectives: It has been shown that subchondral bone mineral density (sBMD) measurement may be a relevant parameter of osteoarthritis (OA) progression. However, factors implicating the reproducibility and contributing to the variability of the measurement have not been fully described. Thus, the aim of this study was to explore the reproducibility of sBMD by Dual energy X-ray Absorptiometry (DXA) and to further examine its sources of variability.
Methods: In this study, short-term, intra and inter-observer reproducibility of sBMD was examined on knee images obtained on DXA scans. The influence of software (lumbar spine and forearm modes), knee positioning (flexion or extension), site and size of regions of interest (ROI) and use of rice, on both lateral and medial tibial sBMD, were assessed. Root mean square coefficient of variation (RMS CV) and least significant changes (LSC) were calculated.
Results: The short-term precision of sBMD ranged between 2.24% and 5.12% for RMS CV and between 0.053 and 0.135 g/cm^2 for LSC. Good intra-observer precision was found for knee flexion conditions whatever the software used (RMS CV ranging from 0.43 to 1.41%). The reproducibility was dependant from the ROI size (the ROI including joint space exhibiting better precision results than ROI including solely the subchondral plate). For a constant size of the ROI, the precision results were site-dependant. Inter-observer RMS CV results ranged from 0.59 to 5.01% according to ROI and software used. For the specific task of monitoring medial sBMD in the ROI including solely subchondral plate, forearm flexion condition produced the highest intra-observer and short-term precision (respectively RMS CV: 0.45% and 2.77%; LSC: 0.013 and 0.080 g/cm^2).
Conclusion: Taking account into the excellent precision of the sBMD measurements expressed as RMS CV with the protocol proposed in the present study, clinical application of these measurements might be envisaged.

Introduction

For a long time, cartilage degeneration was considered as an idiosyncratic feature of osteoarthritis (OA). OA is now recognised as a multifactorial disease involving the whole joint. The role of ligaments, muscles, menisci, synovium, and neural tissue in OA pathophysiology remains not fully studied and is sometimes neglected. Conversely, bone marrow, articular cartilage and subchondral bone are more and more purported to be important in the pathophysiology of OA. The involvement of these last tissues in the progression of the disease has led to the targeting of agents usually devoted to osteoporosis (OP) treatment[1,2]. Recently, it has been shown in a randomised trial that zoledronic acid could reduce knee pain and area bone marrow lesions in patients with clinical knee OA[2]. Strontium ranelate was studied for the treatment of knee OA assuming its effects on bone remodelling[1]. Although there is still debate about the etiopathogenesis and progression of knee OA whether these phenomena are driven by inflammation[3] or by mechanical factors[4], there is an emerging consensus that bone and cartilage cannot be considered as a separate functional unit but conversely are intimately related with biological and mechanical crosstalks[5,6].

On a structural level, knee OA is characterized by subchondral bone sclerosis, osteophyte in growth, subchondral cysts and joint space narrowing. Bone densitometry has been used to characterize the complex relationship between OP and OA[7].

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According to different clinical settings, various results may be observed, for example osteoarthritic human knee joints exhibit an increase in bone volume fraction in patients with total knee replacement surgery[8]. Conversely at the early stages of the disease, studies are scarce due to the paucity of symptoms. However, one cadaveric study reveals rather an osteoporotic phenotype with a trabecular bone network poorly connected[9]. The complex relationship between OP and OA has been studied for a long time, some studies suggesting protective effect of OA for OP[10,11], others relating a rise in fracture risk in postmenopausal women reporting a diagnosis of OA[12]. Assessment of peri-articular bone mineral density (BMD) at the knee joint by Dual energy X-ray Absorptiometry (DXA) was proposed[13] and further evaluated showing its interest in advanced stages of OA[14] and for the prediction of future joint space narrowing[15].

Actually, the osteosclerotic tibial plateau and its changes in knee OA can be considered as a Region Of Interest (ROI) of increased BMD[16], but the femoral condyle has also been identified as a relevant ROI to characterize the OA severity[17]. Although subchondral bone mineral density (sBMD) at the knee has been studied more and more[18], the precision for sBMD has not been fully investigated[19]. The objective of this study was to explore the reproducibility of sBMD by DXA and to further examine its sources of variability[18].

Methods

Subjects

We have conducted a study including 30 subjects (17 women, 13 men). Women had a mean ± SD (range) age of 28.5 ± 8.3 (22–53) years, their height was 165.7 ± 4.6 (158–177) cm, their body weight was 64.8 ± 9.4 (55–95) kg, their body mass index was 23.6 ± 3.2 (20.7–33.7) kg/m^2. Concerning men ($n = 13$), the age was 36.2 ± 11.3 (21–56) years, the height was 178.0 ± 7.0 (162–187) cm, their body weight was 82.4 ± 16.6 (64–127) kg, their body mass index was 25.9 ± 4.4 (21.1–37.5) kg/m^2 of body mass index. The participants were recruited among volunteer employees of the laboratory and the densitometry out patient clinic, with Kellgren–Lawrence scores ≤2. The study was based on the current International Society for Clinical Densitometry recommendation doing precision analyses with at least 30 degrees of freedom (http://www.iscd.org). Subjects were exposed to a 25 μSv dose of radiation equivalent of 3–4 days of natural radiation in France. This study was approved by the local ethics committee of Tours (registration number: 2011-A00322-39/2011-R9).

Radiological measurements

Left knee radiographs were performed in a weight-bearing position with knee flexion as recommended[20]. It has been suggested to use the metatarso-phalangeal (MTP) position in order to obtain a good accuracy and precision[21,22]. The MTP position employed used a specific knee positioner (D3A Medical Systems, Orléans, France); the position was previously described[22]. Images were acquired on high resolution X-ray device (BMA, D3A Medical Systems, Orléans, France) using 65 kV and 20 mAs for all patients. X-rays were blinded scored using the Kellgren–Lawrence classification[23] by the first author (AB) and two senior rheumatologists (EL & SLP).

DXA measurements

The tibial subchondral bone of the knee was scanned by DXA in supine position (Delphi, Hologic, Waltham, MA, USA). All scans were performed on left knees by the same researcher (AB). The device-laser was centered on tibial tuberosity. The four conditions were tested: Lumbar Spine knee extension (LS-ext), Lumbar Spine knee flexion (LS-flex), Forearm knee extension (FA-ext) and Forearm knee flexion (FA-flex). The Lumbar spine software offered 901 μm^2 of area resolution (scan width: 114 mm) whereas the Forearm software offered 426 μm^2 (scan width: 107 mm). For both conditions with knee flexion, the Hologic knee flexor was used in order to obtain a 20° knee flexion. The Hologic foot positioner was used in order to fix a 25° hip rotation for all conditions. This position has been found to optimize the separation of the fibula from the tibia[24].

sBMD values (mean ± SD) were measured on three ROIs, medially and laterally, on each image (Fig. 1) as previously described[19,25,26]. In addition, the range and the difference between the highest and the smallest value in each ROI were calculated for LS-ext, LS-flex and FA-flex.

Design of the study

General design is summarized in Fig. 2. Anthropometric measurements (body weight, height) and knee radiographs were acquired at inclusion as described above. Then, the protocol was divided into five steps:

1) We have evaluated the influence of scan length on the quality of scans (step 1, Fig. 2). Scans were considered as acceptable when the entire bone map was recognized, with no holes. The size of the window and, consequently, the scan length are determinant for bone mass precision as previously observed for lumbar spine and hip DXA assessment[27]. Each image must include at least: tibial tuberosity, femoral condyles and the patella. These anatomical parameters should be scanned in less than 100 mm. Then, we have chosen to use three scan length intervals: 0–100 mm, 101–150 mm and 151–200 mm. These scan lengths were tested on the four conditions: LS-ext, LS-flex, FA-ext and FA-flex for the first 23 voluntary consecutive subjects.
2) In a second time (step 2, Fig. 2), FA-ext condition was excluded according to the step 1 results. The three remaining conditions of position were tested for the short-term reproducibility (i.e., repositioning) and the intra-observer reproducibility (i.e., repeating the placement of ROIs) on the 30 subjects. The short-term reproducibility consisted of three scans per condition acquired for each subject, with an immediate reposition between scans. The intra-observer reproducibility was measured by a single observer (AB) using three sets of analysis on the whole DXA images on the three ROIs.
3) On the step 3, (step 3, Fig. 2) LS-ext condition was excluded for the following reasons:
   a. The knee positioner stabilized the knee and alleviates knee pain for the subject[28]. Moreover, it offered a standardized position, limiting inter-subject variations.
   b. A knee flexion improved the inter-margin alignment (distance between anterior and posterior margins) and facilitated the ROI C drawing, as observed for joint space width measurement in radiograph[29].
   c. The root mean square coefficient of variation (RMS CV) of the ROI C was lower with flexion than extension in both lateral and medial plateaus for intra-observer reproducibility and in medial plateau for short-term reproducibility.

Therefore, both conditions with flexion were used for inter-observer reproducibility (i.e., repeating the placement of ROIs) by the second observer (ED). Thirty images of each condition were randomly selected among all images.

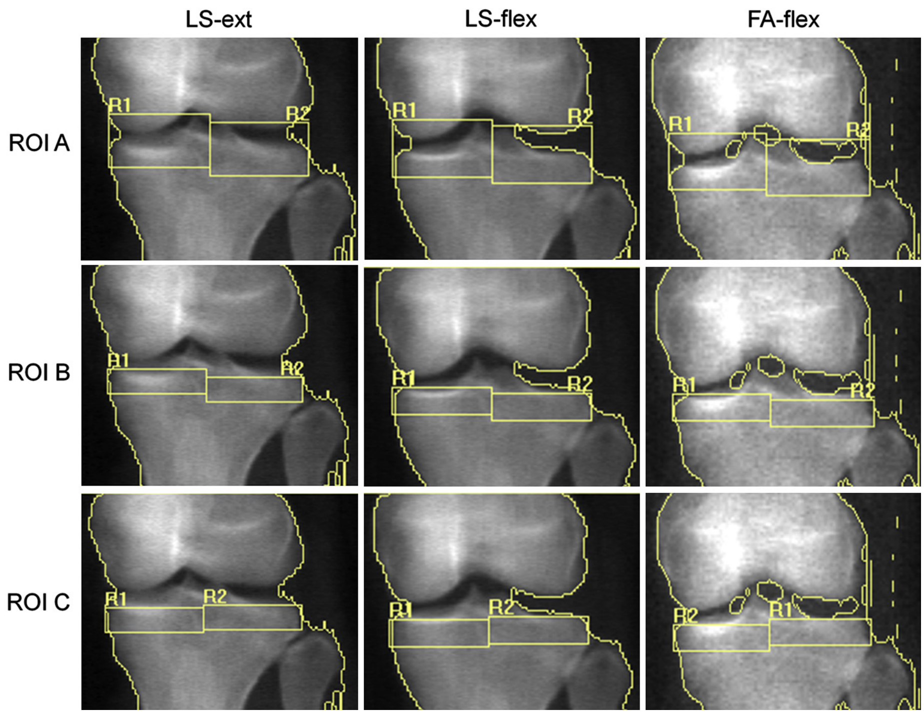

Fig. 1. Representative figures of **ROI A**, **B** and **C** assessed on three conditions in a same subject: **LS-ext** (lumbar spine software with full extended knee); **LS-flex** (lumbar spine software with 20° of knee flexion); **FA-flex** (forearm software with 20° of knee flexion). **R1**: medial ROI, **R2**: lateral ROI. **ROI A**: the top of the ROI A was the highest point of the medial or lateral spine and extended to the edge of the image; either medially or laterally. The ROI has a height of 20 mm and the width of the tibial bone. This ROI included either the medial or lateral intercondylar spine. **ROI B**: the ROI B descended 10 mm down from the highest point of the medial or lateral intercondylar spine. The ROI has a height of 10 mm (10–20 mm beneath the top of the tibial spine). **ROI C**: the top of the ROI C began at the tibial cortical surface and descended from 10 mm; the width of the ROI extended to the edge of the image either medially or laterally. This ROI excluded the joint space.

4) The next step (step 4, Fig. 2), was devoted to sources of variability of sBMD. Ten subjects were scanned with and without rice for the LS-flex condition only. Rice was employed in our study since it has been shown by other authors that the use of rice may compensate the lack of soft tissue surrounding the knee[14,30,31]. Indeed, the presence of air around the knee may disturb the accurate acquisition of bony tissue during scan. Six 1 kg rice bags were positioned circumferentially around the

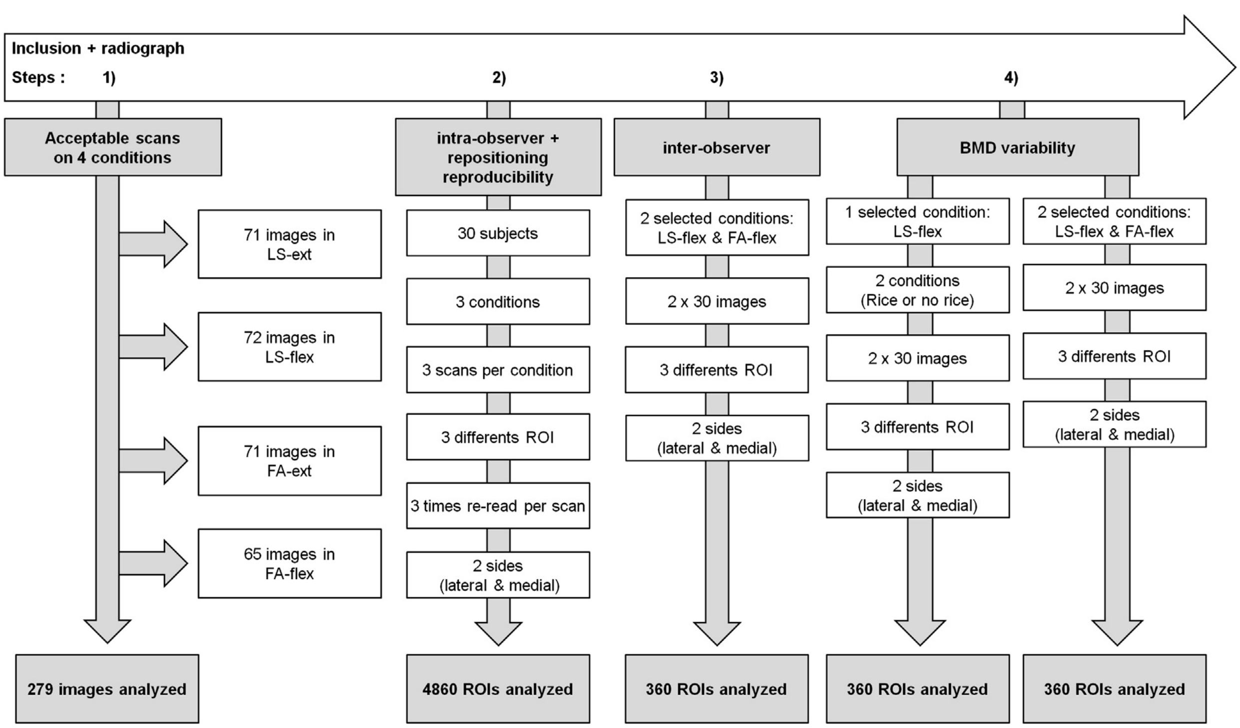

Fig. 2. General design of the study.

knee making sure that there were no pockets of air between the knee and the bags. FA-flex condition was not tested with rice since this software was developed to be insensitive to air presence. However, software-induced sBMD changes were assessed comparing LS-flex to FA-flex for the same 19 subjects (30 scans per condition).

Statistical analysis

All scan data were collected in an Excel (Microsoft office) database table. The acceptable criteria were analysed as a percentage of the acceptable scans relative to total scans. The amplitude of sBMD values was calculated by the highest value obtained for all subjects minus the lowest value. The short-term reproducibility (i.e., immediate reposition) values were analysed using the coefficient of variation (CV)[16,32], the standardized CV (sCV)[33–35], the RMS CV[36–38], the intra-class coefficient of correlation (ICC) calculated with MedCalc software (version 12.3.0.0) with the specific set on "consistency" for one observer as recommended by Fermanian in 2005[39], the smallest detectable difference (SDD)[32] and the least significant changes (LSC)[32], according to the formulae in Appendix.

The intra-observer reproducibility was analysed using the same formulae. The inter-observer reproducibility was evaluated with the same previous formulae and with specific equations for six measures. The ICC test was set with the specific set on "consistency" for two observers and was labelled as ICC1. Two specific equations for two measures were used: RMS CV (2)[36] and ICC (2)[40,41] (see Appendix).

The rice-induced variations were studied by the paired *t*-test. The Gaussian distribution for these parameters was tested by the Kolmogorov–Smirnov test and the variance homogeneity was controlled with the F-test of variances. These tests were achieved on Excel® (Microsoft, Redmond, WA, USA) and on MedCalc® software (version 12.3.0.0).

Results

Scan length

The four initial conditions were tested on the quality of scan depending on its length. The major result was the incapacity to obtain 100% satisfaction with FA-ext condition in all lengths. For the three other conditions, the minimal length for a 100% acceptable scan is ranged between 87 and 118 mm (Table I).

Raw sBMD values

On lateral plateau, we observed a slightly larger range of values with the forearm software than with the lumbar spine software, particularly on ROI C (Table II). Moreover, we observed globally higher amplitude in the lateral compartment than in the medial one. It is noteworthy to associate this latter result with sCV values which are systematically lower in lateral than in medial plateau (Table III).

Short-term reproducibility

The short-term precision of sBMD ranged between 2.24% and 5.12% for RMS CV and between 0.053 and 0.135 g/cm^2 for LSC (Table III). Scores were dependant on ROI size and site, and condition. Globally, ROI A obtained the best results of reproducibility when compared to ROI B and ROI C (RMS CV ranges were respectively as follows: 2.24–3.28% vs 2.63–5.12% and 2.68–3.15%, respectively). Both LS-ext and LS-flex precision were better in lateral plateau compared to medial plateau on all statistical parameters. On the medial plateau, the best RMS CV and LSC scores were found using FA-flex condition in ROI A (respectively 2.35% and 0.061 g/cm^2) and ROI C (respectively 2.77% and 0.080 g/cm^2).

Knee positioner improved tibial medial plateau inter-margin alignment as illustrated in the LS-flex and FA-flex vs LS-ext for a same subject (Fig. 1).

Table I
Acceptable scans in the four conditions with various scan lengths

	Number of measures	Acceptable percentage intervals			Minimal length (mm) for 100%
		0–100 mm	101–150 mm	151–200 mm	
LS-ext	71	63.6%	92.3%	100.0%	118
LS-flex	72	84.1%	100.0%	100.0%	91
FA-ext	71	50.0%	50.0%	78.9%	NA
FA-flex	65	73.2%	100.0%	100.0%	87

LS-ext: Lumbar spine software with full extended knee; LS-flex: Lumbar spine software with 20° of knee flexion; FA-ext: Forearm software with full extended knee; FA-flex: Forearm software with 20° of knee flexion.

Intra-observer reproducibility

Regarding software, we have observed that both LS conditions obtained better reproducibility scores than FA-flex for two ROI on the three analysed. These results were observed with RMS CV, CV, sCV, SDD and LSC (Table IV). Moreover, even if we observed a similar trend with ICC scores (data not shown), the difference observed seem to be related to ROI used.

Indeed, regarding ROI, ROI A obtained better intra-observer reproducibility on both lateral and medial plateaus than ROI B and ROI C concerning RMS CV, SDD and LSC (Table IV), particularly for LS-ext and LS-flex conditions. RMS CV scores ranged from 0.37% to 0.77% on ROI A, from 0.40% to 1.41% on ROI B and from 0.45% to 1.63% on ROI C (Table IV). ROI C measured in FA-flex condition obtained better intra-observer reproducibility on both lateral and medial plateaus than LS-ext and LS-flex whatever the statistical parameters used. Although ICC scores were closed between conditions, we observed a similar trend on ROI C: 0.9994 and 0.9995 with FA-flex (medial and lateral plateau respectively) vs 0.9926 to 0.9969 for LS-ext and LS-flex.

Inter-observer reproducibility

Concerning software, we observed systematic lower precision scores with LS-flex compared to FA-flex (Table V).

When we compared ROIs, ROI A obtained better reproducibility than ROI B and ROI C for LS-flex on medial plateau and for FA-flex on both plateau. When considering LS-flex lateral plateau, the

Table II
Tibial sBMD amplitude

		Amplitude (g/cm^2)		
		ROI A	ROI B	ROI C
MEDIAL	LS-ext	0.553	0.795	0.784
	LS-flex	0.515	0.798	0.727
	FA-flex	0.630	0.748	0.634
LATERAL	LS-ext	0.847	0.773	0.769
	LS-flex	0.729	0.788	0.768
	FA-flex	0.877	0.882	0.918

The amplitude of sBMD values were calculated by the highest value obtained minus the lowest value. Lumbar spine software with full extended knee (LS-ext), lumbar spine software with knee flexion (LS-flex) and forearm software with knee flexion (FA-flex).

Table III
Short-term reproducibility of tibial sBMD

	ROI A						ROI B						ROI C					
	MEDIAL			LATERAL			MEDIAL			LATERAL			MEDIAL			LATERAL		
	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex
RMS CV	2.68%	3.28%	2.35%	2.24%	2.56%	2.41%	3.14%	3.29%	5.12%	2.63%	2.85%	3.46%	3.15%	3.02%	2.77%	2.68%	2.75%	3.14%
CV	2.14%	2.23%	1.93%	1.79%	1.91%	2.08%	2.74%	2.52%	3.95%	2.26%	1.93%	2.82%	2.29%	1.92%	2.23%	1.98%	1.83%	2.52%
sCV	3.19%	3.37%	2.71%	1.98%	2.36%	2.21%	3.14%	2.97%	4.56%	2.39%	2.10%	2.94%	2.90%	2.53%	2.92%	2.40%	2.18%	2.75%
SDD (g/cm^2)	0.053	0.203	0.054	0.052	0.074	0.040	0.101	0.268	0.265	0.074	0.168	0.081	0.119	0.276	0.094	0.070	0.173	0.078
LSC (g/cm^2)	0.064	0.105	0.061	0.063	0.063	0.053	0.088	0.121	0.135	0.075	0.096	0.075	0.095	0.122	0.080	0.074	0.097	0.073

Three conditions were tested: lumbar spine software with extended knee (LS-ext), lumbar spine software with knee flexion (LS-flex) and forearm software with knee flexion (FA-flex). Three different ROIs were tested: ROI A, ROI B and ROI C on both medial and lateral tibial plateaus. Measured parameters were: RMS CV, CV, standardized coefficient of variation (sCV), SDD and LSC.

precision was better for ROI C than ROI A and ROI B (RMS CV: 0.59%, 0.66% and 1.23%, respectively).

The RMS CV (2) showed slightly smaller range than RMS CV: respectively values ranged from 0.58% to 4.75% vs 0.59–5.01%. Similarly, we observed that ICC(2) varied from 0.9853 to 0.9998, whereas ICC(1) varied from 0.9706 to 0.9997.

Rice-induced sBMD differences

sBMD values on both lateral and medial plateaus were significantly lower without rice compared to the use of rice on LS-flex condition (Table VI).

The difference observed first, according to the use or not of rice, second to the condition, was approximately 50 mg/cm^2 (range: 22 mg/cm^2, extreme values: 39–61 mg/cm^2).

Software-induced sBMD differences

ROI A and ROI C on both lateral and medial sBMD were significantly higher with FA-flex compared with LS-flex condition (Table VI). We observed the same trend without rice, that is approximately 30 mg/cm^2 (range: 68 mg/cm^2, extreme values: 9 mg/cm^2 to 77 mg/cm^2) lower sBMD for LS-flex compared to FA-flex.

Discussion

In order to develop sBMD performed at the knee for potential clinical application and research purposes, we examined the reproducibility and the sources of variation of sBMD at the tibial plateau. The main results of this study were the good level of reproducibility of sBMD assessment, whatever the parameter of precision used (mostly under 3.5%, 2.5% and 1.5% for short-term, inter and intra-observer RMS CV, respectively). The reproducibility in ROI A gave the best results with lumbar spine software. However, the reproducibility obtained in medial ROI C was better with forearm software as compared to lumbar spine software.

In the literature, sBMD of the tibia was performed using various devices of different firms: Gammatec[42], Lunar[14,28,30,31,43], Norland[44] and Hologic[15–17,19,24–26]. Most of the researchers have used the lumbar spine software[14,19,24–26,30,31]; this software was not developed for knee analysis and should not measure air around the bone. Lo *et al.* have used rice to improve the grey level of the image and also to get knee flexion and stabilization to reduce motion artefact[14,30,31].

The position of the patient during knee scan is not well defined and there is no consensus about the degree of knee flexion which is recommended. The knee flexion was not always reported in literature. Some authors have chosen a full extended position[28,43,44], whereas others a knee flexion from 5 to 30 degrees[14,15,17,24,30,31]. It seems to be easier to sustain a supine position with a knee flexion, specifically for patients with OA for whom a full extension is very difficult[28]. In addition, we have observed that a knee flexion improved the inter-limb alignment of tibial plateaus, and reduced the RMS CV for ROI C with lumbar spine flexion condition as compared to full extension condition (respectively 1.19% vs 1.63% on medial sBMD and 1.18% vs 1.30% on lateral sBMD). Furthermore, most of the papers aiming at joint space assessment recommend flexion condition to realized conventional knee radiograph[20,22]. Moreover, with full keens extension the forearm software did not obtain 100% of acceptable scans.

It has been shown that a positioning device improves the reproducibility for BMD assessment[45,46]. The position device used in our study permits a constant flexion of the knee at 20° which may limit precision errors in longitudinal studies.

It seems also important to have a hip rotation (10–15 degrees) using Hologic foot positioner in order to separate the tibia and the fibula[24,43].

Alternatively, other software were employed for tibial sBMD measurement. Clarke *et al.* have acquired images with the small animal software on Lunar device[28], the resolution being better than the resolution with the lumbar spine. Using a ROI quite similar to our ROI B, CV ranged from 1.0% to 2.4%, 2.1–5.7% and 7.0% for intra-

Table IV
Intra-observer reproducibility of tibial sBMD

	ROI A						ROI B						ROI C					
	MEDIAL			LATERAL			MEDIAL			LATERAL			MEDIAL			LATERAL		
	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex	LS-ext	LS-flex	FA-flex
RMS CV	0.37%	0.43%	0.76%	0.40%	0.54%	0.77%	1.05%	1.11%	1.41%	0.40%	0.65%	0.73%	1.63%	1.19%	0.45%	1.30%	1.18%	0.49%
CV	0.22%	0.24%	0.33%	0.19%	0.29%	0.36%	0.60%	0.58%	0.74%	0.20%	0.34%	0.49%	0.43%	0.36%	0.24%	0.43%	0.33%	0.31%
sCV	0.24%	0.37%	0.50%	0.19%	0.37%	0.41%	0.60%	0.68%	0.87%	0.20%	0.38%	0.54%	0.46%	0.48%	0.33%	0.47%	0.41%	0.36%
SDD (g/cm^2)	0.002	0.003	0.007	0.002	0.004	0.005	0.013	0.021	0.023	0.002	0.005	0.004	0.043	0.043	0.003	0.020	0.041	0.003
LSC (g/cm^2)	0.012	0.012	0.020	0.010	0.015	0.017	0.028	0.032	0.037	0.011	0.015	0.016	0.051	0.046	0.013	0.035	0.046	0.012

Three conditions were tested: lumbar spine software with extended knee (LS-ext), lumbar spine software with knee flexion (LS-flex) and forearm software with knee flexion (FA-flex). Three different ROIs were tested: ROI A, ROI B and ROI C on both medial and lateral tibial plateaus. Measured parameters were: RMS CV, CV, sCV, SDD and LSC.

Table V
Inter-observer reproducibility of tibial sBMD

	ROI A				ROI B				ROI C			
	MEDIAL		LATERAL		MEDIAL		LATERAL		MEDIAL		LATERAL	
	LS-flex	FA-flex	LS-flex	FA-flex	LS-flex	FA-flex	LS-flex	FA-flex	LS-flex	FA-flex	LS-flex	FA-flex
RMS CV	0.66%	1.52%	0.69%	1.18%	2.74%	5.01%	1.23%	1.75%	1.75%	2.23%	0.59%	1.44%
CV	0.49%	1.13%	0.44%	0.63%	1.95%	3.36%	0.89%	1.26%	1.35%	1.47%	0.43%	0.82%
sCV	0.65%	2.07%	0.53%	1.09%	2.01%	4.83%	0.89%	1.95%	1.53%	2.33%	0.48%	1.28%
SDD (g/cm^2)	0.017	0.030	0.017	0.026	0.065	0.110	0.028	0.036	0.037	0.053	0.015	0.029
LSC (g/cm^2)	0.017	0.036	0.017	0.025	0.070	0.125	0.028	0.036	0.047	0.056	0.014	0.029

Two observers assessed the inter-observer reproducibility on tibial subchondral bone on the two selected conditions lumbar spine software with knee flexion (LS-flex) and forearm software with knee flexion (FA-flex). Three different ROIs were tested: ROI A, ROI B and ROI C on both medial and lateral tibial plateaus. Measured parameters were: RMS CV, CV, sCV, SDD and LSC.

observer, inter-observer and mid-term reproducibility, respectively[28]. Precision scores were not better using small animal than either lumbar spine or forearm software. Indeed, using these two last modes, our CV scores were under 0.75% and less than 3.5% for intra and inter-observer precision, respectively.

Another team has used the hip prosthetic software on an Hologic QDR-4500[17]. According to the manufacturer, it offered an optimized sensitivity for contrast and air, with a resolution similar to lumbar spine software. However, the tibial sBMD reproducibility was not tested in this study[17].

For the first time, we presented the results obtained with the forearm software. We have chosen to use this software for different reasons: (1) this software is calibrated for appendicular skeleton and air is taken into consideration for the grey level, (2) the area resolution (426 μm^2) is the highest as compared to those of either lumbar spine, hip software (901 μm^2) or small animal software (640 μm^2), (3) the scan exam is faster with forearm than with other software. In our study, we have found better precision parameters with the lumbar spine flexion condition mode analysis. However, the reproducibility results obtained with the forearm flexion condition was very close to those obtained with lumbar spine flexion condition (ROI A intra-observer reproducibility expressed as RMS CV: 0.43–0.54% for LS-flex vs 0.76–0.77% for FA-flex). In addition, the best precision on medial ROI C was obtained in FA-flex. Dore *et al.* have reported that sBMD in this ROI predicts cartilage defects[25].

The ROI sizes varied from 1 cm^2 or less[15,42] to about 5 cm^2 for ROIs[14,19,25,26,30,31] located in one plateau[44], until 20 cm^2 or 34 cm^2 in our previous study[47] when ROI were localized in all the epiphysis. No consensus exists on which ROI should be used to measure changes in subchondral bone of the knee with the purpose of monitoring OA progression. It is admitted that higher is the size better is the precision obtained.

Dore *et al.* compared six ROIs in order to determine the most reproducible ROI and their respective interest for knee OA characterization[19]. Three of them obtained acceptable results and were selected for another study by the same authors[25]. ROI A and ROI B sBMD were associated with osteophytes, joint space width and bone marrow lesion[26]. Interestingly, among the three ROIs, only ROI C sBMD predicts OA feature in a prospective cohort[25]. Effectively, ROI C sBMD was associated with cartilage defects using magnetic resonance imaging 2.7 years later[25]. Consequently, ROI C might be a relevant ROI for OA prediction[25].

In this comparative study[19], short-term reproducibility (i.e., repositioning), immediate (i.e., repeating the placement of ROIs) and mid-term (i.e., repeating the placement of ROIs after ~14 days interval) intra-observer reproducibility for all the six ROIs were performed with lumbar spine software condition[19]. Knee degree of flexion was not reported in this work[19]. In this latter work, results were expressed as ICC. We obtained similar results with ICC, values ranging from 0.99 to 1.00 for ROI A, B and C on both LS conditions for intra-observer reproducibility (data not shown). ICC short-term reproducibility ranged from 0.93 to 0.99 in our study (data not shown) and from 0.97 to 0.99 in Dore *et al.* study[19]. The best ROI C intra-observer (1.00) and ROI A and ROI C short-term (0.98–0.99) ICC scores were obtained with the FA-flex condition in our study. All these results may be qualified as very reproducible since ICC $\geq$ 0.91[39]. In the literature, inter-observer reproducibility is not often reported. Hulet *et al.* have investigated intra and inter-observer DXA measurement in tibial sBMD in OA patients[16]. Reproducibility was expressed as CV: 2.8% (intra-observer) and 2.9% (inter-observer) with specific ROIs measuring 7 mm of height. The software used was not reported and knees were fully extended[16]. When considering our whole ROIs and conditions, mean CVs were 0.37% and 1.18% for intra and inter-observer reproducibility, respectively. Our ROIs, initially developed by Dore *et al.*[19], are more reproducible probably due to higher bone surface (about 0.7 cm^2 for Hulet *et al.* vs 5 cm^2 in our work).

There are some limitations in our study. Long-term reproducibility was neither assessed in our study nor in other studies. The longest time interval mid-term reproducibility was 14 days[28]. Our study aimed to explore sBMD variation only with subjects with KL

Table VI
Rice and software-induced differences of tibial sBMD

	ROI A		ROI B		ROI C	
	MEDIAL	LATERAL	MEDIAL	LATERAL	MEDIAL	LATERAL
LS-flex no-rice	0.954 ± 0.108	0.902 ± 0.148	1.154 ± 0.171	0.972 ± 0.152	1.144 ± 0.159	1.006 ± 0.160
LS-flex rice	0.996 ± 0.095*	0.941 ± 0.143*	1.215 ± 0.162*	1.016 ± 0.158*	1.199 ± 0.155*	1.051 ± 0.163*
LS-flex	0.840 ± 0.134	0.792 ± 0.177	0.954 ± 0.193	0.839 ± 0.202	0.985 ± 0.176	0.879 ± 0.212†
FA-flex	0.916 ± 0.167†	0.815 ± 0.207†	0.969 ± 0.207	0.848 ± 0.205	1.018 ± 0.181†	0.902 ± 0.194

Mean values (±SD) of sBMD (g/cm^2) using: 1) LS-flex with rice vs without rice, and 2) lumbar spine software with knee flexion (LS-flex) vs forearm software with knee flexion (FA-flex).

* Significantly different vs NO RICE condition ($P < 0.001$).

† Significantly different vs LS-flex condition ($P < 0.05$).

scores ≤2. We cannot exclude that reproducibility scores might be worse in severe OA patients. It has been demonstrated that OA affects the value of sBMD with higher hip BMD values than in contralateral site[48]. Indeed, it has been shown that lumbar spine osteophytes may affect lumbar spine BMD[49]. Thus, it is plausible that knee osteophytes may influence the raw value of BMD and reproducibility. However, it has been reported that reproducibility was similar or better for OA patients than for healthy patients[15]. Another limitation of our study is that we have not assessed reproducibility at the femoral site. It has been shown that femoral site measurement could be relevant in order to assess sBMD changes associated with OA characterisation[17]. Although the aim of our study was the assessment of precision and not of accuracy, we have observed that presence of air surrounding the knee might lead to under-estimation of sBMD values.

Our results indicated that the use of rice induces changes in sBMD with systematic higher sBMD values (approximately 45–50 mg/cm^2) with lumbar spine mode, and (approximately 30 mg/cm^2) with forearm mode. Some authors have also used rice as an alternative of "soft tissue" around the knee[14,16,30,31]. The different devices and software, the use of rice, the various ROI sizes and sites, and the different patient positions are potential sources of sBMD variability. It explains the difficulties for comparing results between studies and it makes necessary the standardization of knee DXA assessment.

In summary, we have presented for the first time an exhaustive reproducibility study on tibial sBMD on various conditions. We have shown that scan length is a major determinant of acceptable scans. Knee flexion and hip rotation allow the standardization of knee positioning; in addition we have demonstrated that knee flexion improves precision.

Even if precision scores are considered to be good in all conditions, the best results of reproducibility were found using lumbar spine software with knee flexion. We have presented a new application for forearm software which presents a good reproducibility, particularly concerning a specific ROI which has been found to be predictive of medial tibial cartilage defect[25]. However, in this latter work, it is important to precise that the knee was not flexed (but in full extension) and the software employed was not the forearm (lumbar spine). Further studies using this software to measure sBMD in specific region will be useful to evaluate the sensitivity to predict the progression of knee OA.

Contributors

This study was designed by AB and EL. Data were acquired by AB and ED. AB, EL, SLP and SP were involved in the data analysis. AB had full access to all data. AB drafted the paper. All authors revised the manuscript and approved the final draft of the manuscript for submission.

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Conflict of interest

Authors have no conflict of interest.

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Appendix A

$$\mathrm{CV} = \frac{\sum_{i=1}^{n} \frac{\mathrm{SD}_i}{\overline{x_i}}}{n} \times 100$$

$$\mathrm{sCV} = \frac{\mathrm{CV}}{\frac{4\mathrm{SD_{tot}}}{\overline{x}_{\mathrm{tot}}}}$$

$$\mathrm{RMS\ CV} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\frac{\mathrm{SD}_i^2}{\overline{x_i}}} \times 100$$

For three measures:

$$\mathrm{SDD} = 1.96 \times \sum_{i=1}^{n} \mathrm{SD}_i^2$$

And for two measures:

$$\mathrm{SDD} = 1.96 \times \mathrm{SD}\ d_i$$

$$\mathrm{LSC} = 2.77 \times \sqrt{\frac{\sum_{i=1}^{n} \mathrm{SD}_i^2}{n}}$$

$$\mathrm{RMS\ CV(2)} = \frac{\sqrt{\sum_{i=1}^{n} \frac{d_i^2}{2n}}}{\frac{\overline{x_1}+\overline{x_2}}{2}} \times 100$$

$$\mathrm{ICC(2)} = \frac{Sb^2 - Sw^2}{Sb^2 + Sw^2}$$

where Sb^2 and Sw^2 were defined as follows:

$$Sb^2 = \frac{\sum_{i=1}^{n} 2\sqrt{\left(\overline{x_i} - \overline{x_{\mathrm{tot}}}\right)^2}}{n-1}$$

$$Sw^2 = \frac{\sum_{i=1}^{n} \frac{(x_1-x_2)^2}{2}}{n}$$

For all formulae, SD was the standard deviation, i was the number of specimen, d_i was the difference between the first and second measurements, tot (or ij) referred to each measurement (j) of the whole specimens (i), $x_1$was the value measured by observer 1 (AB) and x_2 was the value measured by observer 2 (ED). The bar QUOTE on x indicated the mean of measurements.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.07.009.

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